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(54) Title: STABLE COMPOSITION COMPRISING A NUCLEASE AND A PHOSPHATASE

(57) Abstract: A composition containing a nuclease, preferably Exonuclease I, and a phosphatase, preferably Shrimp Alkaline Phosphatase, wherein the enzymes are combined in a single composition yet each enzyme retains significant functional activity over time. Combining Exonuclease I and Shrimp Alkaline Phosphatase into one composition allows simplified processing of amplified DNA to degrade residual primers and nucleotide triphosphates thereby facilitating subsequent DNA analysis.

1 STABLE COMPOSITION COMPRISING A NUCLEASE AND A PHOSPHATASE

2 This application claims the benefit of U.S. Provisional
3 Patent Application Serial No. 60/190,813, filed March 21,
4 2000.

5 BACKGROUND OF THE INVENTION

6 The invention relates to the field of processing DNA,
7 specifically including amplified DNA, to remove residual
8 primers or other unwanted single-stranded DNA and nucleotide
9 triphosphates prior to performing other operations, such as,
10 but not limited to, DNA sequencing, SNP analysis, or gene
11 expression analysis.

12 Exonuclease I (Exo I) digests single-stranded DNA in a
13 3'→5' direction producing 5' mononucleotides. This enzyme is
14 particularly useful in preparing amplified DNA products, such
15 as PCR products, for sequencing. It degrades residual primers
16 from the amplification reaction that would otherwise be
17 carried over into the sequencing reaction. U.S. Pat. Nos.
18 5,741,676 and 5,756,285 generally disclose methods for DNA
19 sequencing via amplification, both of which are hereby
20 incorporated herein by reference. (See also R.L. Olsen et
21 al., Comp. Biochem. Physiol., vol. 99B, No. 4, pp. 755-761
22 (1991)).

23 Amplification primers carried over into a sequencing
24 reaction could act as sequencing primers and generate
25 sequencing reaction products, thereby creating a background of
26 secondary sequences which would obscure or interfere with
27 observing the desired sequence. Both the concentration and
28 specific activity (purity) of commercially available
29 Exonuclease I may vary over a wide range. Commonly the enzyme
30 is manufactured to a specific activity between 50,000 and
31 150,000 units of enzyme per mg and supplied for the purpose of
32 processing amplified DNA at a concentration around 10 units
33 per microliter. Enzyme with either higher or lower specific

1 activity and either more or less concentrated could be
2 employed in the described applications by suitable alterations
3 in the applied protocol, such as adding less or more volume
4 (or amount) of enzyme, respectively.

5 The storage buffer of commercially available Exonuclease
6 I is: 20 mM Tris-HCl, pH 7.5; 0.5 mM EDTA; 5 mM
7 2-mercaptoethanol; 50 vol.% glycerol, made up in water (major
8 manufacturer and supplier, USB Corporation, Cleveland, Ohio,
9 USA).

10 Alkaline Phosphatases, as exemplified by Shrimp Alkaline
11 Phosphatase (SAP) and Calf Intestinal Alkaline Phosphatase
12 (CIP), catalyze the hydrolysis of 5'-phosphate residues from
13 DNA, RNA, and ribo- and deoxyribonucleoside triphosphates
14 (dNTPs or nucleotide triphosphates). SAP is particularly
15 useful in preparing amplified products, such as PCR products,
16 for sequencing because it can readily be inactivated by heat
17 prior to performing a sequencing reaction. SAP degrades
18 residual dNTPs from the amplification reaction. If residual
19 dNTPs are carried over from the amplification reaction to the
20 sequencing reaction, they add to, and thereby alter, the
21 concentration of dNTPs in the sequencing reaction in an
22 indeterminate and non-reproducible fashion. Since, within
23 narrow limits, high quality sequencing requires specific
24 ratios between the sequencing reaction dNTPs and ddNTPs, an
25 alteration in the concentration of dNTPs may result in faint
26 sequencing reaction signals.

27 The sole manufacturer of SAP has produced enzyme with a
28 wide range of specific activities and concentrations.
29 Examples include batches of enzyme with concentrations ranging
30 from 4.2 units/ μ l to 13.9 units/ μ l with specific activities
31 not being reported. Enzyme with either higher or lower
32 specific activity and either more or less concentrated could
33 be employed in the described applications by suitable
34 alterations in the applied protocol such as adding less or

1 more volume (or amount) of enzyme, respectively. The storage
2 buffer of commercially available Shrimp Alkaline Phosphatase,
3 the preferred enzyme for the above described application, is:
4 25 mM Tris-HCl, pH 7.5; 1 mM $MgCl_2$; 0.1 mM $ZnCl_2$; 50 vol.%
5 glycerol, made up in water (available from USB Corporation,
6 Cleveland, Ohio, USA).

7 Prior to sequencing or other analyses, Exo I and SAP are
8 frequently used to process PCR reaction products. Currently
9 each enzyme is supplied in its own storage buffer as described
10 above. In a recommended procedure (see "PCR Product Pre-
11 Sequencing Kit" protocol booklet, USB Corporation) one
12 microliter of each enzyme preparation is independently added
13 (via pipetting) to 5 microliters of PCR reaction product. In
14 this application multiple pipetting steps potentially can
15 introduce significant experimental error, both determinant and
16 indeterminant, into subsequent sequencing measurements.
17 Furthermore, the ratio of Exo I to SAP can vary significantly
18 among subsequent experiments due to delivery of imprecise
19 relative volumes of each of the enzyme preparations to
20 subsequent batches of amplified DNA.

21 Historically, a stable composition comprising both
22 enzymes in fixed proportion has not been commercially
23 produced. It may have been thought that the $MgCl_2$ and $ZnCl_2$,
24 both present in the commercial SAP storage buffer, were
25 incompatible with the EDTA present in the commercial Exo I
26 storage buffer. EDTA is a chelating agent that reacts
27 strongly with Mg^{2+} and Zn^{2+} ions. When mixed together such that
28 the EDTA is in molar excess, the EDTA effectively sequesters
29 Mg^{2+} and Zn^{2+} ions thereby preventing these ions from
30 interacting with any protein(s) present in the solution. As a
31 class, alkaline phosphatases are considered to be multimeric,
32 metallo-enzymes that require a divalent ion, frequently Zn^{2+} ,
33 for structural stability and activity.

34 Consequently, there is a need in the art for a stable

1 composition comprising both enzymes in a single delivery
2 vehicle. Preferably, such a stable composition will enjoy a
3 long shelf life, each enzyme retaining a significant
4 proportion of its original functional activity over time.

5 SUMMARY OF THE INVENTION

6 A composition comprising a nuclease and a phosphatase is
7 provided. The composition is substantially free from the
8 presence of amplified deoxyribonucleic acid. The phosphatase
9 in the composition retains at least 50% of its functional
10 activity when the composition is stored at 4°C for 24 hours.
11 A method of degrading preselected nucleic acids present in a
12 sample of material is also provided. The method comprises the
13 step of contacting the sample with a composition comprising a
14 nuclease and a phosphatase.

15 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE
16 INVENTION

17 As used herein, when a range such as 5-25 or 5 to 25 or
18 between 5 and 25 is given, this means preferably at least 5
19 and, separately and independently, preferably not more than
20 25.

21 As used herein, and in the appended claims, when the
22 concentration of a component is provided as a volume/volume
23 percent (% v/v), this means that that component is present by
24 volume in a proportion relative to the total volume of the
25 composition (including all of its constituent components)
26 equal to the stated percent for the specific component. By
27 way of example, a composition with 50% v/v of glycerol is
28 composed of a volume of glycerol equal to one half (or 50%) of
29 the total volume of the composition including all of its
30 components (including glycerol and water if present). In such
31 a composition, concentrations reported in molarity (M) are
32 based upon the total volume of the composition including all
33 of its components.

1 As used herein, one unit of nuclease (e.g. Exo I) enzyme
2 is that amount of nuclease enzyme required to catalyze the
3 release of 10 nmol of acid-soluble nucleotide from denatured
4 DNA in 30 minutes at 37°C under standard conditions.

5 As used herein, one unit of phosphatase (e.g. SAP) enzyme
6 is that amount of phosphatase enzyme required to catalyze the
7 hydrolysis of 1 μ mol of p-nitrophenylphosphate per minute in
8 glycine/NaOH buffer (pH 10.4) at 37°C.

9 As used herein, the term "functional activity" generally
10 refers to the ability of an enzyme to perform its designated
11 function as described below. As used herein, the functional
12 activity of nuclease (e.g. Exo I) is qualitatively defined in
13 terms of the ability of nuclease enzyme to degrade residual
14 PCR primers from PCR amplified DNA to a level low enough so as
15 not to materially interfere with subsequent sequencing
16 reactions or other applications. The functional activity of
17 nuclease is measured for Exo I using the following
18 methodology. 1 μ l of a solution containing Exo I is added to
19 5 μ l of PCR amplified DNA and the mixture incubated at 37°C
20 for 15 minutes. The reaction is terminated by heating to 80°C
21 for 15 minutes. The treated DNA is then used as a template in
22 a standard sequencing reaction, such as the USB T7-Sequenase
23 V2.0 PCR Product Sequencing Kit, and the quality of the
24 sequencing ladder examined to determine the effectiveness of
25 degrading residual primers from the amplified DNA. Exo I, as
26 commercially supplied by USB Corporation for this application,
27 can be used between 0.5 and 20 units, preferably 1-15 units,
28 more preferably at about 10 units per 5 μ l reaction product in
29 standard pre-sequencing processing of PCR amplification
30 product. Quantitatively, the functional activity and half-
31 life of Exo I and other nucleases of the invention are
32 ascertained after a specified period of storage at a specified
33 temperature as described in the following paragraph.

34 Original Exo I composition containing 10 units Exo I/ μ l

1 is prepared at time zero, and a serial dilution performed,
2 such that the concentration of enzyme in each successive
3 dilution is one half that of the prior dilution, for a total
4 of preferably 5 dilutions plus the original undiluted
5 composition. This results in the following: original
6 undiluted composition, one half dilution, one quarter
7 dilution, one eighth dilution, one sixteenth dilution, and one
8 thirty-second dilution. Presuming no change in activity, the
9 enzyme equivalents per microliter of Exo I composition in each
10 respective dilution (beginning with the undiluted composition)
11 are: 10 units Exo I; 5 units Exo I; 2.5 units Exo I, 1.25
12 units Exo I, 0.625 units Exo I; and 0.3125 units Exo I;
13 corresponding to the undiluted composition, as well as
14 dilutions equal to one half, one fourth, one eighth, one
15 sixteenth, and one thirty-second the concentration of the
16 undiluted composition. At time zero, 1 μ l of each of the
17 above is separately delivered to a separate 5 μ l sample of a
18 control PCR reaction product (which has been pretreated or is
19 being co-treated to materially degrade the dNTPs) containing
20 residual DNA primers to be degraded prior to sequencing, and
21 the enzyme is permitted to degrade the residual primers. The
22 sequencing is then performed and the sequence ladders (six in
23 this example) compared. In looking at the sequence ladders or
24 lanes, the first dilution where the sequencing ladder exhibits
25 material secondary and/or multiple lane signals compared to
26 the primary sequencing signal indicates that the enzyme
27 activity dropped off at that dilution. This is referred to as
28 the "drop-off dilution". This is used as a measuring stick or
29 baseline for determining, at a subsequent point in time, the
30 half-life and functional activity of the enzyme. At each of
31 several subsequent points in time after storage at a specified
32 temperature, e.g. 24 hours, 2, 3, 5, 7, 14, 21, 30, 60, 90,
33 etc., days, a similar serial dilution analysis is performed on
34 a portion of the original stored composition, and the "drop-

1 off dilution" is again ascertained. The first time that the
2 "drop-off dilution" shifts from one dilution (for example, the
3 one sixteenth dilution) to the prior dilution (for example,
4 the one eighth dilution) indicates the point in time that the
5 half-life of the nuclease enzyme has been reached. For
6 example, assume a serial dilution analysis was conducted every
7 day and it took 7 days for the drop-off dilution to shift from
8 the one sixteenth dilution to the one eighth dilution. This
9 indicates that at 7 days, the enzyme has lost one half of its
10 functional activity, because now, for the first time, it takes
11 twice as much enzyme activity (the one eighth dilution is
12 twice as concentrated as the one sixteenth dilution) to
13 achieve the same result, i.e. full or material degradation of
14 residual primer. Since it takes twice as much enzyme
15 activity, the enzyme has reached its half-life.

16 For example, an original Exo I composition containing 10
17 units Exo I per μ l is prepared and subject to serial dilution
18 analysis as described above. It is found that the drop-off
19 dilution is the one thirty-second dilution. The composition
20 is then stored at 4°C for a period of time, say one week. The
21 stored composition is again subjected to serial dilution
22 analysis, and the drop-off dilution remains the one thirty-
23 second dilution. Serial dilution analyses are subsequently
24 performed at 2, 3, 4, 5, etc., weeks, and it is found at the
25 5th week test that, for the first time, the drop-off dilution
26 is the one sixteenth dilution. This indicates that the half-
27 life point has been reached. In this example, it can be seen
28 that the half-life point was reached between the fourth and
29 fifth weeks. Thus in this example, the nuclease enzyme in the
30 composition retained at least 50% of its functional activity
31 when the composition was stored for four weeks at 4°C.

32 As used herein, the functional activity of phosphatase
33 (e.g. SAP) is qualitatively defined in terms of the ability of
34 phosphatase enzyme to degrade residual PCR nucleotide

1 triphosphates from PCR amplified DNA to a level low enough so
2 as not to materially interfere with subsequent sequencing
3 reactions or other applications. The functional activity of
4 phosphatase is measured for SAP using the following
5 methodology. 1 μ l of a solution containing SAP is added to 5
6 μ l of PCR amplified DNA and the mixture incubated at 37°C for
7 15 minutes. The reaction is terminated by heating to 80°C for
8 15 minutes. The treated DNA is then used as template in a
9 standard sequencing reaction, such as the USB T7-Sequase
10 V2.0 PCR Product Sequencing Kit, and the quality of the
11 sequencing ladder examined to determine the effectiveness of
12 degrading residual nucleotide triphosphates from the amplified
13 DNA. If residual nucleotide triphosphates in PCR amplified
14 DNA are not effectively degraded, the nucleotide triphosphates
15 from the PCR reaction will alter the ratio of dNTPs/ddNTPs in
16 the sequencing reaction causing faint signals. Independently
17 formulated SAP, as commercially supplied by USB Corporation
18 for this application, can be used to degrade residual
19 nucleotide triphosphates in PCR amplified DNA between 0.1 and
20 5 units, preferably 1-3 units, more preferably at about 2
21 units per 5 μ l reaction product in standard pre-sequencing
22 processing of PCR amplification product. Quantitatively, the
23 functional activity and half-life of SAP and other
24 phosphatases of the invention are ascertained via periodic
25 serial dilution analyses similarly as explained above with
26 respect to Exo I. An original SAP composition containing 2
27 units SAP per μ l is prepared, and 1 μ l of the original
28 undiluted SAP composition and 5 serial dilutions thereof are
29 delivered separately to separate 5 μ l samples of a control PCR
30 reaction product (preferably having been pretreated or being
31 co-treated to degrade residual primers) having residual
32 nucleotide triphosphates to be cleaned up, and the enzyme is
33 permitted to degrade the nucleotide triphosphates. The
34 sequencing is then performed and the sequence ladders compared

1 as before. In looking at the sequence ladders or lanes, the
2 first dilution where the first 50 bases of a DNA sequencing
3 ladder having more than 200 discernable bases are materially
4 fainter than in the prior dilution indicates that the enzyme
5 activity dropped off at that dilution. This is referred to as
6 the "drop-off dilution", and is used as a measuring stick or
7 baseline for determining, at subsequent points in time, the
8 half-life and functional activity of the enzyme. At each of
9 several subsequent points in time after storage at a specified
10 temperature, e.g. 24 hours, 2, 3, 5, 6, 14, 21, 30, 60, 90,
11 etc., days, a similar serial dilution analysis is performed on
12 a portion of the original stored composition, and the "drop-
13 off dilution" is again ascertained. Half-life for SAP is then
14 determined similarly as explained above with respect to Exo I.

15 For example, an original SAP composition containing 2
16 units SAP per μ l is prepared and subject to a serial dilution
17 analysis as described above. It is found that the drop-off
18 dilution at time zero is the one thirty-second dilution. The
19 composition is then stored at 4°C for a period of time, say
20 one week. The stored composition is then subjected to another
21 serial dilution analysis, and the drop-off dilution remains
22 the one thirty-second dilution. Serial dilution analyses are
23 subsequently performed at 2, 3, 4, 5, etc., weeks, and it is
24 found at the 5th week test that, for the first time, the drop-
25 off dilution is the one sixteenth dilution. In this example,
26 it can be seen that the half-life point was reached between
27 the fourth and fifth weeks. Thus in this example the
28 phosphatase enzyme in the composition retained at least 50% of
29 its functional activity when the composition was stored for
30 four weeks at 4°C.

31 Characteristics of the Preferred Compositions

32 The present invention relates to a single composition
33 comprising both a nuclease and a phosphatase, wherein less
34 than 50%, preferably less than 40%, preferably less than 30%,

1 preferably less than 20%, preferably less than 10%, of the
2 functional activity of each and/or either enzyme is lost per
3 24 hours, more preferably per week, even more preferably per
4 month, and most preferably per 4 months, when held or stored
5 under a specified condition such as -20°C, 0°C, +4°C, or room
6 temperature (e.g. +20°C).

7 The phosphatase in the composition preferably retains at
8 least 50% of its functional activity when said composition is
9 stored at 4°C for 24, more preferably 36, more preferably 48,
10 more preferably 60, more preferably 72, more preferably 96,
11 hours. The nuclease in the composition preferably retains at
12 least 50% of its functional activity when said composition is
13 stored at 4°C for 2, more preferably 3, more preferably 5,
14 more preferably 7, more preferably 9, more preferably 12, more
15 preferably 14, days. The invented composition is preferably
16 substantially free from the presence of deoxyribonucleic acid,
17 nucleic acid, amplified DNA, nucleotide triphosphates,
18 oligonucleotides, and primers, each of which could interfere
19 with the composition's performance.

20 Preferably, the nuclease is heat-labile, preferably
21 single-stranded exonuclease, preferably Exonuclease 7 or RecJ,
22 most preferably Exo I, and the phosphatase is preferably heat-
23 labile, preferably eukaryotic phosphatase, preferably
24 bacterial or animal phosphatase, preferably mammal
25 phosphatase, most preferably SAP. The invented composition
26 preferably is formulated in such a manner that when an aliquot
27 of 2 µl of the composition is contacted with 5 µl of PCR
28 reaction product (DNA that was amplified by standard PCR
29 techniques), the residual primers and nucleotide triphosphates
30 are effectively inactivated or degraded by being decreased to
31 a level that allows effective sequencing of the amplified
32 product. The amounts and concentrations of the Exo I, SAP and
33 other materials may vary depending upon the specific nature
34 and amount of the amplified DNA product, the nature and amount

1 of residual primers and nucleotide triphosphates, the time and
2 temperature of the processing reaction, and the sequencing
3 method used. Embodiments of the invention also allow for
4 adding different volumes or proportions of the combined
5 composition as needed to achieve the desired result. Further
6 embodiments allow the composition containing nuclease, such as
7 Exo I, and phosphatase, such as SAP, to be dehydrated or dried
8 (or optionally lyophilized), thus comprising at most 10 wt.%
9 water, and these concentrated or dried forms to be contacted
10 with the amplified DNA.

11 The invention provides a nuclease and a phosphatase in a
12 single composition. The composition can be used for degrading
13 residual materials present in the product of a nucleic acid
14 synthesis reaction, examples of which are referenced or
15 described in this paragraph. The method involves contacting
16 (for example, mixing) the reaction product with the
17 composition. The composition can be used for cleaning up or
18 degrading residual primers and residual nucleotide
19 triphosphates, preferably after a DNA or RNA amplification
20 reaction, preferably a PCR or RT-PCR amplification reaction,
21 alternatively an isothermal amplification reaction. The
22 composition can also be used for cleaning up a nucleic acid
23 (preferably DNA) replication reaction, such as primer-
24 initiated RNA or DNA synthesis. After such degrading of the
25 residual materials in the reaction product, the cleaned-up
26 reaction product can be used in subsequent analyses, such as
27 DNA sequencing, less preferably SNP (Single Nucleotide
28 Polymorphism) analysis (which is a way of determining single
29 nucleotide differences), other genetic analyses (including
30 gene expression) or other analyses of nucleic acids where
31 cleanup of residual primers, residual oligonucleotides and/or
32 residual nucleotide triphosphates is useful, such as analysis
33 of multiple base additions, deletions or differences.

34 The invented composition can also be used, with or

1 without additional nucleases and/or phosphatases, to act as a
2 selective and/or all-purpose clean-up composition to clean up
3 samples other than amplification reaction products, such as a
4 biological sample such as biopsy materials, blood samples,
5 bodily fluids, or intermediates used in the production of
6 biological materials. In such a case the composition
7 containing a nuclease and a phosphatase would degrade
8 preselected nucleic acids present in the sample of material.
9 The sample could be material, such as biopsy material,
10 isolated from biological material, such as a human body.

11 With respect to the disclosure of this invention the
12 referenced stability generally relates to compositions held in
13 either liquid or dried states. However, it is recognized that
14 combinations of Exo I and SAP can be stored frozen. In this
15 case if frozen quickly enough and held at a low enough
16 temperature compositions of Exo I and SAP could be held with
17 potentially little reduction in functional activity or
18 performance for extended periods of time such as at least 6,
19 12, 24, 36, 60 or 100 months. Preferably the invented
20 composition retains at least 10, 20, 30, 40, 50, 60, 70, 80
21 and/or 90 % of its functional activity for each enzyme
22 following storage of the composition for 24 hours, or 2, 3, 4,
23 5, 8, 10, 15, 20, 30, 40, 60, 80, 100, 120, 150, 180, 210,
24 240, 300, 360, 500, 1000, 1500, 2000 and/or 3000 days at 25°C,
25 20°C, 18°C, 10°C, 4°C, 0°C, -10°C, -20°C, -30°C, -40°C, -60°C,
26 -80°C, -100°C, -150°C or -190°C. The invented compositions are
27 packaged, stored, shipped and used as known in the art.

28 Preferred Compositions

29 The only necessary components of the invented composition
30 are the enzymes, that is, the nuclease and the phosphatase.
31 The other components described herein are preferred but are
32 optional. The nuclease is preferably Exonuclease I (Exo I)
33 and the phosphatase is preferably alkaline phosphatase,
34 preferably Shrimp Alkaline Phosphatase (SAP) as indicated

1 above. The combination of enzymes can be supplied in dried
2 form or, more preferably, in a liquid, preferably in an
3 aqueous solution. Preferred aqueous solutions are described
4 herein. Less preferably, the enzymes can be supplied in more
5 concentrated solutions, such as solutions (with or without the
6 optional components) which are at least 2, 3, 4, 5, 6, 8, 10,
7 15, 20, 30, 50, 80, 100, 150, 200, 300, 500, 800, 1,000,
8 2,000, 5,000, 8,000, or 10,000 times more concentrated than
9 the solutions described herein, or concentrated all the way to
10 dryness. Diluted solutions can also be provided. In the
11 invented composition, any preferred or less preferred
12 concentration or range of any component can be combined with
13 any preferred or less preferred concentration or range of any
14 of the other component or components; it is not required or
15 necessary that all or any of the components or concentrations
16 or ranges be that which is most preferred.

17 Preferably, the composition is a liquid, preferably
18 aqueous, combination of a nuclease and a phosphatase
19 (preferably an alkaline phosphatase), preferably Exo I and
20 SAP, where the Exo I to SAP unit ratio is between 1:5000 and
21 5000:1, more preferably between 1:500 and 500:1, even more
22 preferably between 1:50 and 50:1 and most preferably between
23 1:10 and 10:1 with a total protein concentration ranging from
24 1 µg/ml to 200 mg/ml, more preferably 10 µg/ml to 100 mg/ml,
25 even more preferably 100 µg/ml to 50 mg/ml and most preferably
26 between 1.0 mg/ml and 10 mg/ml. With such a combination of
27 Exo I and SAP the units of Exo I contacted with 5 µl PCR
28 amplified DNA could range from 0.01 to 100 units of Exo I,
29 more preferably 0.1 to 30 units of Exo I, even more preferably
30 1 to 15 units of Exo I and most preferably 10 ±4 units of Exo
31 I, the 5 µl PCR amplification reaction product is also
32 preferably contacted with 0.01 to 100 units of SAP, more
33 preferably 0.1 to 10 units of SAP, even more preferably 0.5 to
34 5 units of SAP and most preferably 2 ±1 units of SAP.

1 Optionally, other alkaline phosphatases, such as calf
2 intestinal alkaline phosphatase, may be used in place of the
3 SAP. The concentration of nuclease in the invented
4 composition is preferably at least 0.01, 0.1, 1, 2, or 5 units
5 of nuclease enzyme per microliter. The concentration of
6 phosphatase in the invented composition is preferably at least
7 0.01, 0.1, 1, 2, or 5 units of phosphatase enzyme per
8 microliter.

9 In the invented composition preferably the pH is between
10 4.0 and 12.0, more preferably between pH 6.0 and 10.0, more
11 preferably between 7.0 and 9.0, more preferably less than 8,
12 more preferably between 7 and 8, and most preferably pH 7.5
13 ± 0.2 or pH 7.5 ± 0.3 , preferably controlled by a buffer. The
14 invented composition may optionally and preferably contain a
15 buffer at a concentration of zero to 250 mM, more preferably
16 between 5 mM to 100 mM, even more preferably between 15 mM to
17 50 mM and most preferably 25 \pm 5 mM, preferably of Tris-HCl,
18 preferably at pH 7.5 to pH 8.5 or the pH ranges mentioned
19 above. Other buffers may be used such as, but not limited to:
20 organic buffers such as MOPS, HEPES, TRICINE, etc., or
21 inorganic buffers such as Phosphate or Acetate. Buffers or
22 other agents may be added to control the pH of the solution
23 thereby increasing the stability of the enzymes.

24 The invented composition may optionally and preferably
25 contain a reducing agent such as but not limited to:
26 dithiotreitol (DTT) or 2-mercaptoethanol; preferably zero to
27 100 mM, more preferably 0.1 mM to 50 mM, even more preferably
28 0.5 to 10 mM and most preferably 1.0 \pm 0.2 mM. Reducing agents
29 may be added to limit enzyme oxidation that might adversely
30 affect stability of the enzymes.

31 The invented composition may optionally and preferably
32 contain monovalent ions such as, but not limited to: Na⁺, K⁺,
33 Li⁺, Cl⁻, Br⁻ or acetate (HCO₂⁻) at a concentration of zero to
34 500 mM, more preferably 0.5 mM to 100 mM, even more preferably

1 1 mM to 50 mM and most preferably 1 to 10 mM. The presence of
2 monovalent ions can help prevent protein precipitation which
3 might lead to inactivation; addition of other compounds such
4 as chelating agents frequently lead to the addition of trace
5 amounts of monovalent ions.

6 The invented composition may optionally and preferably
7 contain a complexing or chelating agent such as, but not
8 limited to, $\text{Na}_2\text{-EDTA}$ or $\text{Na}_2\text{-EGTA}$ at a concentration of zero to
9 100 mM, more preferably 0.05 to 10 mM, even more preferably
10 0.1 to 2 mM, and most preferably 0.5 ± 0.1 mM. Chelating
11 agents are frequently added to protein solutions to sequester
12 metal ions which if present can catalyze changes in amino acid
13 side chain chemistry and under certain conditions cause breaks
14 in the amino acid backbone of enzymes, thereby decreasing
15 activity.

16 The invented composition may optionally contain an amino
17 acid based carrier or stabilizer such as, but not limited to,
18 bovine serum albumin and Poly L-lysine, preferably at a
19 concentration between zero and 100 mg/ml, more preferably
20 between 0.01 and 10 mg/ml and most preferably between 0.1 and
21 1.0 mg/ml.

22 The invented composition may optionally contain divalent
23 ions such as but not limited to: Zn^{2+} , Mg^{2+} , Co^{2+} , Mn^{2+} and/or
24 Ca^{2+} , preferably at a concentration between zero and 200 mM,
25 more preferably between zero and 20 mM, more preferably
26 between 0.0001 mM and 5 mM and most preferably 0.002 to 1.0
27 mM. Divalent ions are preferred or required for effective
28 enzyme activity of some proteins, such as phosphatases. Trace
29 amounts of divalent ions may be present as a result of the
30 addition of other substances to the composition; the normal
31 composition of SAP contains both Zn^{2+} and Mg^{2+} which may
32 accompany the enzyme into the composition.

33 The invented composition may optionally contain
34 detergents (singly or in combination) such as, but not limited

1 to, non-ionic, ionic or zwitterionic detergents added to
2 stabilize the enzymes or enhance performance. For example
3 Nonidet P40, Triton X100 or Tween 20 between zero and 20% v/v,
4 more preferably between 0.01% and 5% v/v, and most preferably
5 between 0.1% and 1.0% v/v. Similarly SDS, singly or in
6 combination with other detergents, may be added between zero
7 and 5% v/v, more preferably between 0.0001% and 1% v/v, and
8 most preferably between 0.005% and 0.1% v/v.

9 The invented composition may optionally contain other
10 chemicals added that enhance performance such as, but not
11 limited to, DMSO between zero and 50% v/v, more preferably
12 between 0.001% and 10% v/v, most preferably between 0.01% and
13 1% v/v.

14 The invented composition may optionally contain a dextran
15 such as Dextran T-10 or Dextran T500 or other polysaccharide
16 between zero and 50% v/v, more preferably between 0.1% and 10%
17 v/v and most preferably between 1% and 5% v/v.

18 The invented composition may optionally and preferably
19 contain an enzyme stabilizer or a material that inhibits ice
20 formation such as, but not limited to, glycerol, ethylene
21 glycol or glycine, preferably glycerol, preferably at a
22 concentration of zero to 99% v/v, more preferably 1% to 75%
23 v/v, more preferably 5% to 65% v/v, more preferably 20% to 60%
24 v/v, more preferably 35% to 58% v/v, and most preferably 50
25 \pm 5% v/v.

26 The invented composition may optionally contain mono- or
27 disaccharide such as glucose or maltose that may stabilize the
28 enzymes or facilitate the composition of a dry embodiment.
29 The mass of the mono- or disaccharide is preferably at least
30 zero, 0.1, 1, 10, 100, 1000 or 10,000, or not more than 10 or
31 100 or 1000 or 10,000, times the mass of the protein in the
32 composition.

33 The most preferred compositions according to the
34 invention are described below as Compositions D and E.

1 Composition D is preferred for manual pipetting operations,
2 and composition E is preferred for automated pipetting
3 operations. Where composition D is used, preferably 2 μ l of
4 composition D are combined with 5 μ l of PCR reaction product
5 to effectively degrade residual primers and nucleotide
6 triphosphates prior to sequencing. Where composition E is
7 used, preferably 5 μ l of composition E are combined with 5-25
8 μ l, preferably 5 μ l, of PCR reaction product to effectively
9 degrade residual primers and nucleotide triphosphates prior to
10 sequencing or other analyses. Whether using composition D or
11 E, it is preferred that 10 units of Exo I and 2 units of SAP
12 are delivered to 5 μ l of product containing residual primers
13 and/or nucleotide triphosphates to be degraded.

14 Further aspects of the present invention will now be
15 demonstrated, and the invention will be better understood in
16 conjunction with the following examples, which describe
17 preferred embodiments of the invention. The following
18 examples are provided by way of illustration and not
19 limitation, and it should be understood that other nuclease-
20 and phosphatase-containing compositions comprising other
21 combinations and concentrations of optional components are
22 possible and intended.

23 EXAMPLES

24 In conjunction with the following experiments, 5 separate
25 nuclease/phosphatase compositions were prepared, and are
26 generally referred to herein as Compositions A through E. The
27 compositions and component concentrations of each composition
28 are provided below.

29 Composition A was prepared as an aqueous composition with
30 the following components: 10 units/ μ l of Exonuclease I; 2
31 units/ μ l of Shrimp Alkaline Phosphatase; 25 mM Tris-HCl, pH
32 7.5; 0.5 mM $\text{Na}_2\text{-EDTA}$; 1 mM DTT; 50% v/v glycerol, made up in
33 water. Concentrated stocks of Exo I and SAP were dialyzed

1 against 25 mM Tris-HCl, pH 7.5; 0.5 mM Na₂-EDTA; 1 mM DTT; 50%
2 v/v glycerol. Following dialysis the enzymes were combined in
3 Composition A so that each microliter of Composition A
4 contained 10 units of Exo I and 2 units of SAP. Enzyme
5 activity assays as well as enzyme functional activity were
6 measured, as indicated in table 1, after the composition was
7 stored at -20°C, 4°C and +25°C for various lengths of time.

8 **Composition B** was prepared as an aqueous composition with
9 the following components: 10 units/μl of Exonuclease I; 2
10 units/μl of Shrimp Alkaline Phosphatase; 25 mM Tris-HCl, pH
11 7.5; 100 μg/ml bovine serum albumin; 1 mM DTT; 1 mM MgCl₂; 0.1
12 mM ZnCl₂; 50% v/v glycerol, made up in water. Concentrated
13 stocks of Exo I and SAP were dialyzed against 25 mM Tris-HCl,
14 pH 7.5; 100 μg/ml bovine serum albumin; 1 mM DTT; 1 mM MgCl₂;
15 0.1 mM ZnCl₂; 50% v/v glycerol. Following dialysis the enzymes
16 were combined in Composition B so that each microliter of
17 Composition B contained 10 units of Exo I and 2 units of SAP.
18 Enzyme functional activity was measured, as indicated in table
19 1, after the composition was stored at -20°C, 4°C and +25°C
20 for various lengths of time.

21 **Composition C** was prepared as an aqueous composition with
22 the following components: 10 units/μl of Exonuclease I; 2
23 units/μl of Shrimp Alkaline Phosphatase; formulated into 50 mM
24 Tris-HCl, pH 8.3; 0.5 mM Na₂-EDTA; 1 mM DTT; 0.5% v/v Tween 20;
25 0.5% v/v Nonidet P-40, 50% v/v glycerol, made up in water.
26 The composition was made by mixing the appropriate amount of
27 Exo I and SAP, in their commercially available storage
28 buffers, into Composition C. This composition thus contained
29 small amounts of MgCl₂ and ZnCl₂ derived from the commercial
30 SAP composition. Functional activity was measured, as
31 indicated in table 1, after the composition was stored at -
32 20°C, 4°C or 25°C for various lengths of time.

33 **Composition D** was prepared as an aqueous composition with
34 the following components: 5 units/μl of Exonuclease I; 1

1 unit/ μ l of Shrimp Alkaline Phosphatase; formulated into 25 mM
2 Tris-HCl, pH 7.5; 0.5 mM $\text{Na}_2\text{-EDTA}$; 1mM DTT; 50% v/v glycerol.
3 This composition was made by mixing the appropriate amount of
4 Exo I and SAP, in their commercially available storage
5 buffers, into Composition D. Composition D thus contains
6 traces of MgCl_2 and ZnCl_2 derived from the commercial SAP
7 composition, and 2-mercaptoethanol derived from the Exo I
8 composition. In order to deliver 10 units of Exo I and 2
9 units of SAP, a working volume of 2 μ l of this enzyme mixture
10 was used. Enzyme functional activity was measured, as
11 indicated in table 1, after the composition was stored at
12 -80°C , -20°C , 4°C , and 25°C for various lengths of time. A
13 freeze and thaw experiment was also performed.

14 Composition E was prepared as an aqueous composition with
15 the following components: 2 units/ μ l of Exonuclease I; 0.4
16 units/ μ l of Shrimp Alkaline Phosphatase; formulated into 25 mM
17 Tris-HCl, pH 7.5; 0.5 mM $\text{Na}_2\text{-EDTA}$; 1 mM DTT; 50% v/v glycerol.
18 This composition was made by mixing the appropriate amount of
19 Exo I and SAP, in their commercially available storage
20 buffers, into Composition E. Composition E thus contains
21 traces of MgCl_2 and ZnCl_2 derived from the commercial SAP
22 composition, and 2-mercaptoethanol derived from the Exo I
23 composition. In order to deliver 10 units of Exo I and 2
24 units of SAP, a working volume of 5 μ l for this enzyme mixture
25 is a convenient volume for addition to PCR reaction mixtures
26 by robotic pipettors. Enzyme functional activity was
27 measured, as indicated in table 1, after the composition was
28 stored at -20°C for various lengths of time.

29 The functional activity of each of the above
30 nuclease/phosphatase compositions was determined at the
31 various stated temperatures and after the stated elapsed times
32 as described above and further as described below. A sample
33 of each composition was removed as appropriate and a serial
34 1:1 dilution made into the respective composition, such that

1 the concentration of enzyme in each successive dilution was
2 one half that of the prior dilution. For Compositions A-C,
3 presuming no change in activity, these enzyme equivalents per
4 volume addition to the PCR reaction product (per μ l of the
5 enzyme composition) were: 10 units Exo I with 2 units SAP; 5
6 units Exo I with 1 unit SAP; 2.5 units Exo I with 0.5 units
7 SAP; 1.25 units Exo I with 0.25 units SAP; 0.625 units Exo I
8 with 0.125 units SAP; and 0.3125 units Exo I with 0.0625 units
9 SAP. These amounts thus represented the respective undiluted
10 compositions, as well as dilute compositions diluted to one
11 half, one fourth, one eighth, one sixteenth, and one thirty-
12 second the concentration of the respective undiluted
13 compositions.

14 These serial dilutions resulted in concentration of
15 enzyme that paralleled those made with untreated Exo I and SAP
16 stock enzyme. Performance of the enzyme dilutions was then
17 examined by the standard performance assay employing the USB
18 T7-Sequenase V 2.0 PCR Product Sequencing Kit and using 1 μ l
19 of diluted composition per assay for Compositions A, B and C;
20 2 μ l of diluted composition per assay for Composition D; and 5
21 μ l of diluted composition for Composition E.

22 The functional activity of nuclease and phosphatase
23 enzymes was determined as described above. The half-life of
24 each composition was that point in time when either the
25 nuclease (Exo I) or the phosphatase (SAP) in the composition
26 reached its half-life, ie., had lost at least 50% of its
27 functional activity. Tabular results are presented in table 1
28 of Example 1 below, with additional results and detailed
29 explanation following in Examples 2-5.

30

1 EXAMPLE 1: SUMMARY OF STABILITY DATA FOR COMBINED COMPOSITIONS2 A-E AT TEMPERATURES RANGING FROM -80°C TO +25°C

3 Table 1: Stability of Exo I and SAP in Compositions A - E

Temp. (°C)	Activity Half-Life				
	Composition A	Composition B	Composition C	Composition D	Composition E
25	> 12 hours	-	<< 1 hour	> 12 hours	-
4	> 3 days	-	-	> 3 days	-
-20	> 4 months	> 5 weeks	< 2 days	> 4 months	> 5 weeks
-80	-	-	-	No detectible loss after 8 weeks	-

4 The activity half-life as expressed in table 1 is that
5 duration of storage required to observe a 50% reduction in
6 functional activity of either the Exo I or the SAP in the
7 composition.

8 EXAMPLE 2: STABILITY AT -20°C OF EXONUCLEASE I AND SHRIMP9 ALKALINE PHOSPHATASE ENZYMES IN A COMBINED COMPOSITION

10 Unexpectedly after 8 weeks of storage at -20°C, Compositions
11 A, B and D showed significant retention in functional activity
12 of either the Exonuclease I or shrimp alkaline phosphatase as
13 compared to their respective control enzymes. Even more
14 unexpectedly, upon formulation over a 100% gain in SAP
15 functional activity was observed in the test of Compositions A
16 and D, the compositions containing an excess of EDTA. In this
17 test when only 0.25 units of commercially formulated SAP (a
18 1/8 dilution) were used to react amplified PCR DNA, the bottom
19 of the DNA sequence ladder was faint. This indicates that
20 when this amount of SAP was used not all the residual dNTPs
21 from the amplification reaction were degraded. When SAP was
22 combined with Exo I in either Composition A or D, a strong
23 sequencing reaction was still obtained when only 0.125 units
24 of SAP (a 1/16 dilution) were used to react with the amplified
25 PCR DNA product. This result was particularly surprising

1 because published characterizations of SAP (Oksen, et.al.,
2 1991) would lead one to expect the enzyme to lose nearly all
3 its activity. Composition B exhibits an unexpected retention
4 in functional activity (see table 1), but did not exhibit the
5 unexpected increase in activity exhibited by Compositions A
6 and D. Composition E also unexpectedly exhibited significant
7 retention in activity (see table 1).

8 EXAMPLE 3: STABILITY AT +4°C OF EXONUCLEASE I AND SHRIMP
9 ALKALINE PHOSPHATASE ENZYMES IN A COMBINED COMPOSITION

10 Unexpectedly, considerable functional activity of SAP in
11 Composition A and Composition D was retained following storage
12 at +4°C with less than 50% of its functional activity being
13 lost in three days. (See table 1).

14 EXAMPLE 4: STABILITY AT +25°C OF EXONUCLEASE I AND SHRIMP
15 ALKALINE PHOSPHATASE ENZYMES IN A COMBINED COMPOSITION

16 Unexpectedly, considerable functional activity of SAP in
17 Composition A as well as Composition D was retained following
18 storage at +25°C with as much as 25% of the original
19 functional activity being retained after one day of storage at
20 +25°C. This retention of activity appears to be even greater
21 than that reported for SAP when stored in its normal,
22 commercially available composition ("Shrimp Alkaline
23 Phosphatase", Monograph, Biotec-Mackzymal AS, Tromso, Norway).

24 EXAMPLE 5: STABILITY AT -80°C OF EXONUCLEASE I AND SHRIMP
25 ALKALINE PHOSPHATASE ENZYMES IN COMBINED COMPOSITION D

26 Upon thawing after 8 weeks of storage at -80°C, Composition D
27 exhibited no detectable loss of functional activity of either
28 Exonuclease I or Shrimp Alkaline Phosphatase.

29 In addition to the most preferred components and
30 component concentrations described above, combined
31 nuclease/phosphatase compositions according to the invention
32 can be prepared using other, less preferred components and
33 component concentrations. Table 2 summarizes various

1 components and component concentrations that can be used in
 2 the invented composition. In table 2, any preferred or less
 3 preferred or more preferred concentration or range of any
 4 component can be combined with any preferred or less preferred
 5 or more preferred concentration or range of any of the other
 6 components; it is not required or necessary that all or any of
 7 the concentrations or ranges come from the same column.

8 Table 2: Further Preferred Components for the Invented
 9 Composition

Component/Property	Most Preferred	Less Preferred	Less Preferred	Least Preferred
Exo I (units to be added to 5 µl PCR reaction product)	10 ±4 units	1-15 units	0.1-30 units	0.01-100 units
SAP (units to be added to 5 µl PCR reaction product)	2 ±1 units	0.5-5 units	0.1-10 units	0.01-100 units
Composition pH	7.5 ±0.2	7.0-9.0	6.0-10.0	4.0-12.0
Buffer (Tris-HCl, MOPS, HEPES, TRICINE, etc.)	25 ±5 mM Tris-HCl	15-50 mM	5-100 mM	0-250 mM
Reducing Agents (DTT, B-ME)	1.0 ±0.2 mM DTT	0.5-10 mM	0.1-50 mM	0-100 mM
Monovalent Ions (Na ⁺ , K ⁺ , Li ⁺ , Cl ⁻ , etc.)	Trace	1-50 mM	0.5-100 mM	0-500 mM
Complexing/Chelating Agents (Na ₂ -EDTA, Na ₂ -EGTA, etc.)	0.5 ±0.1 mM Na ₂ -EDTA	0.1-2.0 mM	0.05-10 mM	0-100 mM
Amino Acid Based Carrier (Bovine Serum Albumin, Poly l-lysine, etc.)	0	0-1.0 mg/ml	0-10 mg/ml	0-100 mg/ml
Divalent Ions (Zn ²⁺ , Mg ²⁺ , Co ²⁺ , etc.)	0.002-1.0 mM	0.0001-5 mM	0-20 mM	0-200 mM

Nonionic Detergents (Nonidet P40, Triton X100, Tween 20, etc.)	0	0.1%-1% v/v	0.01%-5% v/v	0-20% v/v
Zwitterionic Detergents (CHAPS, CHAPSO, etc.)	0	0.01%-1% v/v	0.005%-5% v/v	0-20% v/v
Ionic Detergents (SDS, etc)	0	0.005%-0.1% v/v	0.00001%-1% v/v	0-5% v/v
Other chemicals such as DMSO	0	0.01%-1% v/v	0.001%-10% v/v	0-50% v/v
Polysaccharide/Dext ran	0	1%-5% v/v	0.1%-10% v/v	0-50% v/v
Stabilizer (glycerol, ethylene glycol, etc)	50% \pm 5% v/v	5%-65% v/v 30%-70% v/v 40%-60% v/v	1%-75% v/v 25%-75% v/v	0-99% v/v 10%-90% v/v 20%-80% v/v
Mono- or disaccharide (glucose, maltose, etc.)	0	10-10,000 X protein mass	1-100 X protein mass	0.1-10 X protein mass
Water	Balance water or 50% \pm 5% v/v	30%-70% v/v 40%-60% v/v	25%-75% v/v 20%-80% v/v 10%-90% v/v	3%-99% v/v 1%-99.5% v/v

1 Although the hereinabove described embodiments of the
2 invention constitute the preferred embodiments, it should be
3 understood that modifications can be made thereto without
4 departing from the scope of the invention as set forth in the
5 appended claims.

WHAT IS CLAIMED IS:

- 1 1. A composition comprising a nuclease and a
2 phosphatase, said composition being substantially free from
3 the presence of amplified deoxyribonucleic acid.
- 1 2. A composition according to claim 1, said composition
2 being substantially free from the presence of nucleic acid.
- 1 3. A composition according to claim 1, said composition
2 being substantially free from the presence of nucleotide
3 triphosphates and primers.
- 1 4. A composition according to claim 1, said composition
2 comprising an effective amount of shrimp alkaline phosphatase.
- 1 5. A composition according to claim 4, said composition
2 comprising an effective amount of Exonuclease I.
- 1 6. A composition according to claim 1, wherein said
2 phosphatase is alkaline phosphatase.
- 1 7. A composition according to claim 1, wherein said
2 nuclease is a single-stranded exonuclease.
- 1 8. A composition according to claim 1, said composition
2 further comprising an effective amount of a buffering agent.
- 1 9. A composition according to claim 8, wherein said
2 buffering agent is Tris-HCl.
- 1 10. A composition according to claim 1, said composition
2 having a pH of 7 to 8.
- 1 11. A composition according to claim 1, said composition
2 further comprising an effective amount of a reducing agent.
- 1 12. A composition according to claim 1, said composition
2 further comprising an effective amount of a chelating agent.

1 13. A composition according to claim 1, said composition
2 further comprising at least 20 volume percent of a stabilizer
3 selected from the group consisting of glycerol, ethylene
4 glycol and glycine.

1 14. A composition according to claim 1, wherein said
2 nuclease is present in said composition in a concentration of
3 at least 0.1 units of enzyme per microliter.

1 15. A composition according to claim 1, wherein said
2 phosphatase is present in said composition in a concentration
3 of at least 0.1 units of enzyme per microliter.

1 16. A composition according to claim 1, said composition
2 being capable, upon being added to the product of a PCR
3 amplification reaction, of effectively degrading residual
4 primers and permitting effective DNA sequencing.

1 17. A composition according to claim 1, said composition
2 being capable, upon being added to the product of a PCR
3 amplification reaction, of effectively degrading residual
4 nucleotide triphosphates and permitting effective DNA
5 sequencing.

1 18. A composition according to claim 1, wherein said
2 composition consists essentially of said nuclease and said
3 phosphatase.

1 19. A composition comprising a nuclease and a
2 phosphatase, said phosphatase in said composition retaining
3 at least 50% of its functional activity when said composition
4 is stored at 4°C for 24 hours.

1 20. A composition according to claim 19, said nuclease
2 in said composition retaining at least 50% of its functional
3 activity when said composition is stored at 4°C for 3 days.

1 21. A method of degrading preselected nucleic acids
2 present in a sample of material, the method comprising the
3 step of contacting said sample with a composition comprising a
4 nuclease and a phosphatase.

1 22. A method according to claim 21, wherein said sample
2 is material isolated from biological material.

1 23. A method according to claim 21, wherein said
2 preselected nucleic acids present in said sample of material
3 are residual materials present in a product of a nucleic acid
4 synthesis reaction and wherein the method comprises the step
5 of contacting said synthesis reaction product with said
6 composition comprising said nuclease and said phosphatase.

1 24. A method according to claim 23, wherein said
2 composition is substantially free from the presence of
3 amplified deoxyribonucleic acid.

1 25. A method according to claim 23, wherein said
2 synthesis reaction product contains residual primers and
3 wherein said nuclease degrades said residual primers present
4 in said reaction product.

1 26. A method according to claim 23, wherein said
2 synthesis reaction product contains residual nucleotide
3 triphosphates and wherein said phosphatase degrades said
4 residual nucleotide triphosphates present in said reaction
5 product.

1 27. A method according to claim 23, wherein said
2 synthesis reaction is primer-initiated DNA synthesis.

1 28. A method according to claim 23, wherein said
2 synthesis reaction is a DNA amplification reaction.

1 29. A method according to claim 23, wherein said

2 synthesis reaction is a PCR amplification reaction.

1 30. A method according to claim 23, wherein said
2 synthesis reaction is an isothermal amplification reaction.

1 31. A method according to claim 23, wherein said
2 synthesis reaction is an RT-PCR amplification reaction.